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Development and Application of Efficient Forward and Reverse Genetic Tools in *Acetobacterium woodii*

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Acetobacterium woodii represents one of the most studied and, therefore, best understood anaerobic acetogens. Currently the CO₂CHEM consortium (comprising the Universities of Ulm, Frankfurt and Nottingham, together with the Novo Nordisk Foundation Center for Biosustainability, Siemens and LanzaTech) seek to exploit its potential as a chassis for chemical and fuel manufacture through ERA-IB funding. Essential to this goal are the availability of effective gene tools for genome engineering, both by directed and random mutagenesis. These systems have been implemented through adherence to our recently published roadmap for gene system development [1].

As a first step we established a high efficiency transformation system for *A. woodii*, allowing for the subsequent use of suicide vectors to create a *pyrE* knockout strain. This uracil auxotroph provides the background for an ACE knockout system which has been exemplified through phenotypic studies of substrate pathway gene deletions.

An orthogonal expression system based on the unique *Clostridium difficile* sigma factor TcdR, has been shown to act exclusively on the toxin *tcdA/B* gene promoters and so are not recognized by *E. coli* RNA polymerase. To ensure expression solely in the target host (*A. woodii*), the *tcdR* gene was introduced into the *A. woodii* genome using an ACE cargo vector at the *pyrE* locus. This allowed for the use of *tcdB* to drive a mariner transposon system on a suicide vector to create a library of mutants for both phenotypic and high-throughput genome sequencing studies; further increasing our understanding of this important chassis strain.

[1] Minton NP, Ehsaan M, Humphreys CM, Little GT, Baker J, Henstra AM, Liew F, Kelly ML, Sheng L, Schwarz K, Zhang Y. A roadmap for gene system development in *Clostridium*. *Anaerobe*. 2016; **41**:104-112.